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मानक

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“जानने का अधिकार, जीने का अधिकार”

Mazdoor Kisan Shakti Sangathan

“The Right to Information, The Right to Live”

“पुराने को छोड़ नये के तरफ”

Jawaharlal Nehru

“Step Out From the Old to the New”

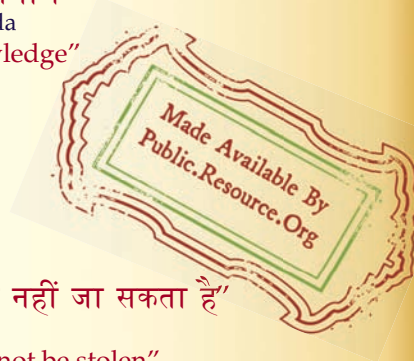
IS 6582 (Part 2) (2001, Reaffirmed 2007): Bio-Assay Methods for Evaluating Acute Toxicity of Industrial Effluents and Waste Waters, Part 2: Using Toxicity Factor to Zebra Fish (Brachydanio Rerio). ICS 13.060 : 13.060.30



“ज्ञान से एक नये भारत का निर्माण”

Satyanarayan Gangaram Pitroda

“Invent a New India Using Knowledge”



“ज्ञान एक ऐसा खजाना है जो कभी चुराया नहीं जा सकता है”

Bhartrhari—Nitiśatakam

“Knowledge is such a treasure which cannot be stolen”

IS 6582 (Part 2) : 2001

REAFFIRMED

SEP 2007

भारतीय मानक

औद्योगिक बहिःस्राव एवं अपशिष्ट जल के लिए अत्याधिक
आविषालुपता के मूल्यांकन की बायो-ऐसे पद्धति
भाग 2 जेबरा फिश में आविषालुपता कारक का
प्रयोग करके (ब्रेकाईडेनियो, रेट्रो)
(पहला पुनरीक्षण)

Indian Standard

BIO-ASSAY METHOD FOR EVALUATING ACUTE
TOXICITY OF INDUSTRIAL EFFLUENTS AND
WASTE WATERS

PART 2 USING TOXICITY FACTOR TO ZEBRA FISH (BRACHYDANIO RERIO)

(First Revision)

ICS 13.060; 13.060.30

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

May 2001

Price Group 3

FOREWORD

This Indian Standard (First Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Water Environment Protection Sectional Committee had been approved by the Chemical Division Council.

The bio-assay method, specified in IS 6582 : 1971 [now proposed to be revised and numbered as IS 6582 (Part 1)], is based on determination of LC_{50} , that is, concentration of sample (of effluent/waste water) on semi-logarithmic scale at which 50 percent of fish could survive. However, this method requires a large number of fish and takes longer time to give result.

Keeping the above in view, for regulatory purposes, present method which is based on toxicity factor (T_F), that is, minimum number of times an effluent/waste water is required to be diluted for obtaining no mortality for zebra fish, has been developed. Reproducibility and validity of this method have been found to be acceptable by Central Pollution Control Board, after testing on treated/untreated pesticides/industrial effluents, collected from different parts of the country.

The proposed method, using toxicity factor (T_F) will lead to lower concentration level compared to the LC_{50} ; however, the former is preferable as it gives toxicity potential of waste water in terms of dilution required to acceptable permissible level.

The composition of the committee responsible for the preparation of this standard is given in Annex A.

In reporting the result of a test or analysis made in accordance with this standard if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

AMENDMENT NO. 2 MARCH 2007
TO
IS 6582 (PART 2) : 2001 BIO-ASSAY METHOD FOR
EVALUATING ACUTE TOXICITY OF INDUSTRIAL
EFFLUENTS AND WASTE WATERS

PART 2 USING TOXICITY FACTOR TO ZEBRA FISH
(BRACHYDANIO RERIO)

(First Revision)

(Page 1, clause 5) — Substitute the following for the existing text:

5 SAMPLING

5.1 Point of Sampling

In those cases where the effluent at a specific point is to be tested, the question of choosing the point of sampling does not arise. However, where the composition of an effluent as finally discharged by the sewage treatment plant is to be ascertained, the point of sampling shall be the final outlet of the treatment plant.

5.2 Frequency of Sampling

When it is required to find out variations in the composition of the effluent during a specified period, such as that of peak discharge, the samples shall be taken at short and appropriate intervals, say, every 5, 10, 15 or 30 min and analysed. To study the average conditions over a cycle of operations or a period (usually 24 hours) or during the daily working period of the treatment plant, the collection of composite sample shall be adopted. The composite sample shall be made by collecting at appropriate intervals samples from the common channel or drain at a point where the flow of the effluent is likely to be most representative of the entire volume, and mixing. The volume of the individual samples shall be a fixed proportion of the volume of the effluent flowing at that time. The interval should depend upon the frequency of variation in the nature of the effluent and the volume of flow. Care shall be taken to take the samples in such a way as to maintain the true proportion of suspended solids. Samples shall not be taken by skimming the top or scraping the bottom. A point about one-third of the way from the bottom shall normally be selected. The samples

Amend No. 2 to IS 6582 (Part 2) : 2001

shall be drawn gently without unnecessary aeration. In most cases, collection of samples every hour would be sufficient.

5.3 Sampling Instrument

Porcelain-lined or enamelled pails, in which the lining is unbroken, or glass vessels shall be used for taking samples. The vessels used for taking the sample shall be wide-mouthed and small enough for the contents to be transferred quickly to the sample container without leaving behind any deposit or scum. Automatic sampling devices, if available, may be used.

5.3.1 Each individual sample shall be deposited in a receptacle of sufficient size to hold the entire composite sample. Clean and dry carboys, other large glass containers or enamelled buckets with lids without chipping may be used for pooling the sample.

5.4 Sample Containers

5.4.1 The quantity of sample required for analysis shall be taken from the composite sample after thorough mixing in order to keep the solids in suspension.

5.4.2 The sample for analysis shall be drawn in clean glass-stoppered bottles, which shall be rinsed with a portion of the sample. New bottles shall be washed with acid and thoroughly rinsed with distilled water before being brought into use. About 2 to 3 litres of the sample will be required for analysis. The bottle containing the final sample shall be filled so that a small air bubble is present after closure to prevent leakage or even breakage arising from any subsequent changes in temperature. The stopper shall be firmly inserted and, if the sample is to be transported some distance, tied down to keep in position.

5.4.3 The label on the bottle shall bear the name of the sampling authority, details of the type of sample, place, date and time of sampling.

5.5 Preservation of Samples

5.5.1 The samples shall be kept at a low temperature (about 4°C) during collection and thereafter.

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5.5.2 No single method of preservation is applicable for the sample for all the tests. The analysis shall be carried out, preferably, immediately after collection. Storage at 3° to 4°C in a well insulated ice box or refrigerator is the best way to preserve most samples till the next day. Where chemical preservatives are used as specified for individual tests, these shall be added to each portion of sample taken for the particular test and not to the entire sample.

(CHD 32)

Reprography Unit, BIS, New Delhi, India

**AMENDMENT NO. 1 DECEMBER 2002
TO
IS 6582 (PART 2) : 2001 BIO-ASSAY METHOD
FOR EVALUATING ACUTE TOXICITY OF INDUSTRIAL
EFFLUENTS AND WASTE WATERS**

**PART 2 USING TOXICITY FACTOR TO ZEBRA FISH
(BRACHYDANIO RERIO)**

(First Revision)

[Page 3, clause 7.3(f)] — Substitute '48 h' for '96 h'.

(CHD 32)

Reprography Unit, BIS, New Delhi, India

Indian Standard

BIO-ASSAY METHOD FOR EVALUATING ACUTE TOXICITY OF INDUSTRIAL EFFLUENTS AND WASTE WATERS

PART 2 USING TOXICITY FACTOR TO ZEBRA FISH (BRACHYDANIO RERIO)

(First Revision)

1 SCOPE

The standard prescribes an alternative method for determination of acute lethal toxicity of waste water and industrial effluents to zebra fish (*Brachydanio rerio*) under specified conditions.

2 REFERENCES

The following standards contain provisions which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standard indicated below:

IS No.	Title
1070 : 1992	Reagent grade water (<i>third revision</i>)
4733 : 1972	Methods of sampling and test for sewage effluents (<i>first revision</i>)

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1 Dilution Water

The dilution water shall have a pH of 7.8 ± 0.2 and a calcium hardness of approximately 200 mg/l expressed as CaCO_3 . It shall be prepared in accordance with 7.1.

3.2 Test Water

It is the mixture of waste water and dilution water, which is used for testing.

3.3 Dilution Factor (T)

Dilution Factor (T) is the numerical expression of volumetric proportion of the waste water in test water. 'T' denotes the toxicity unit.

3.4 Toxicity Factor, T_F

T_F is the lowest Dilution Factor T of the Test Water at which all fish survive. T_F denotes the toxicity for fish. This is a dimensionless toxicity unit, which is used as

standard parameter for compliance monitoring of waste water discharges. Higher the T_F , the greater is the toxicity of the tested waste water.

3.5 Death of Fish

A fish is declared dead if it does not move when touched.

3.6 Range Finding Test

Range finding test is a preliminary test for samples with unknown toxicity to determine the range of dilution within which the T_F value is expected.

3.7 Standard Test

Standard test is the confirmatory test performed to determine the T_F value, under strict compliance with the procedure prescribed in this method.

4 GENERAL PRINCIPLE

4.1 Fish are affected by toxic substances chiefly in following two ways:

- Epithelia absorb toxic substances, getting damaged in this process, for instance, the gills stick together or get congested with mucus and get destroyed.
- Besides the above immediate effects, harmful substances are adsorbed through the gills, skin or intestine, thereby impairing physiological functions. These effects may ultimately lead to the death of fish.

4.2 These toxic effects are determined in terms of the death of the test organism in standard dilution series of waste water samples with standard dilution water.

5 SAMPLING

Representative samples of the material shall be drawn as prescribed in 2 of IS 4733.

6 MATERIAL

6.1 Test-Fish Species

Brachydanio rerio (Hamilton Buchanan), having a size

of 30 ± 5 mm, corresponding to approximately 0.2 to 0.3 g of mass.

6.2 Stock of Fish

6.2.1 The fish shall be kept at temperature of approximately 25°C in aerated chlorine free potable water of roughly similar characteristics as the dilution water. The population density of fish shall not exceed 1 g per litre.

6.2.2 The daily illumination shall be in the range of 12 to 16 h. The stock shall be kept on a normal diet. The fish shall be free of manifest diseases or visible malformations. The minimum acclimatisation period shall be 10 days prior to test under conditions of water quality and illumination similar to those used in the test. Mortality shall not exceed 1 percent per week.

6.2.3 For each test, fish shall be selected from the same stock tank, the population of which is under conditions of water quality and illumination similar to those applied in the test.

6.3 Equipment

All equipment shall be of inert material, preferably of stainless steel or borosilicate glass.

6.3.1 *Glass Beaker* — 3 l capacity.

6.3.2 *Measuring Pipettes* — 5 and 10 ml.

6.3.3 *Volumetric Pipettes* — 10, 20 and 50 ml.

6.3.4 *Volumetric Flask* — 100 ml and 1 litre.

6.3.5 *Measuring Cylinders* — 100 ml and 1 litre.

6.3.6 *Beakers* — 100, 200, 500 ml and 1 litre.

6.3.7 *Thermometer*

6.3.8 *Oxygen Meter*

6.3.9 *pH Meter*

6.3.10 *Conductivity Meter*

6.3.11 *Tanks for Fish Stock and Dilution Water*

6.3.12 *Water Baths*

6.3.13 *Aquaria*

6.3.14 *Aerators*

6.3.15 *Thermostats*

6.3.16 *Air and Water Filter with Activated Charcoal*

6.3.17 *Handnets*

6.4 Reagents

6.4.1 *Calcium Chloride* ($\text{CaCl}_2, 2 \text{H}_2\text{O}$)

6.4.2 *Magnesium Sulphate* ($\text{MgSO}_4, 7 \text{H}_2\text{O}$)

6.4.3 *Sodium Bicarbonate* (NaHCO_3)

6.4.4 *Potassium Chloride* (KCl)

6.5 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in the tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

7 PROCEDURE

7.1 Preparation of Dilution Water

7.1.1 Dilution water is prepared by mixing 25 ml each of the following four stock solutions and diluting to 1 litre with water. Thereafter the pH is adjusted using sodium hydroxide or hydrochloric acid solution.

7.1.1.1 *Calcium chloride solution* — Dissolve 11.76 g of calcium chloride dihydrate ($\text{CaCl}_2, 2\text{H}_2\text{O}$) in water and dilute to 1 litre.

7.1.1.2 *Magnesium sulphate solution* — Dissolve 4.93 g of magnesium sulphate heptahydrate ($\text{MgSO}_4, 7\text{H}_2\text{O}$) in water and dilute to 1 litre.

7.1.1.3 *Sodium bicarbonate solution* — Dissolve 2.59 g of sodium bicarbonate (NaHCO_3) in water and dilute to 1 litre.

7.1.1.4 *Potassium chloride* — Dissolve 0.23 g of potassium chloride (KCl) in water and dilute to 1 litre.

7.1.2 Aeration of the Dilution Water

The dilution water shall be aerated for 24 h prior to the test.

7.2 Preparation of Test Solutions

7.2.1 Range Finding Test

In case the range of toxicity of an effluent is unknown, a range-finding test should be performed prior to the standard test to determine the concentration range within which 0 percent and 100 percent mortality is observed within 24 h. For example, in case of an effluent with unknown toxicity, the following dilution series with a dilution factor of 10 may be used :

Dilution Factor(T)	Ratio of Waste Water to Test Water (by Volume)	Parts of Dilution Water to be Added to one Part of Waste Water (by Volume)
1	1:1	0
10	1:10	9
100	1:100	99
1 000	1:1 000	999

7.2.2 Standard Test

7.2.2.1 The test water is prepared by adding the effluent to the dilution water in a rounded logarithmic (geometric) progression scale as given below:

Dilution Factor (T)	Ratio of Waste Water to Test Water (by Volume)	Parts of Dilution Water to be Added to one Part of Waste Water (by Volume)
1	1:1	0
2	1:2	1
4	1:4	3
8	1:8	7
16	1:16	15
32	1:32	31
64	1:64	63
125	1:125	124
250	1:250	249
500	1:500	499
1 000	1:1 000	999

7.2.2.2 Up to five consecutive concentrations have to be selected based on previous experience with regard to potential toxicity of the effluent. Each test vessel is filled with 2 litres of test water. One control vessel containing only dilution water is provided for each test. The test may be performed with one replicate.

7.3 Test Conditions

Following test conditions shall be maintained:

- 24 h prior to the test, feeding shall be stopped.
- Fish for a single test, shall be selected from a tank with population of the same stock.
- After obtaining the correct temperature, 5 fishes shall be placed in each of the vessels. The fishes shall be selected randomly from the stock population and distributed at random in the various vessels with the help of a small fine-mesh dip net of soft inert material.
- Any fish dropped or otherwise harmed during the transfer shall be discarded. All the fish, for a single test shall be introduced to test vessels within a period of 30 min.
- The samples and test solutions shall not be aerated or treated; else extreme BOD content or extreme pH values may influence the result.
- The duration of the test shall be 96 h.
- Count of the dead fish in each vessel shall be taken each after 2 h, 6 h, 24 h and 48 h; the dead fish shall be removed from the vessels.

- In case of fish dying in the control vessel, the test shall be discarded and fresh testing shall be repeated.
- The dissolved oxygen concentration, the pH and temperature in each vessel shall be measured at the beginning of the test and each after 24 h and 48 h.
- The handling of fish, solutions and all test procedures shall be carried out in premises free of harmful concentrations of vapours. Any disturbances that may change the behaviour of fish shall be avoided.
- All tests shall be carried out under normal laboratory illumination with natural photo period.
- The temperature shall be maintained at $25 \pm 1.0^\circ\text{C}$.

8 CALCULATIONS

8.1 The dilution factor of the test solution with the highest concentration of effluent in which all fish survive shall be recorded in rounded numbers as T_F .

Example : If $T_F = 8$, it shows that all or some of the fish die in the test solution for a T value, less than 8 (1 part waste water + 7 parts of dilution water) and all are alive in test solutions for a T value of 8 and above, after 48 h.

9 VALIDITY

9.1 The results shall be considered valid if the following requirements are met.

9.1.1 The T_F value of the reference chemical for each stock of fish should be in agreement with results obtained previously in the same laboratory, as a reference material potassium dichromate is used. In dilution water containing 100 mg/l potassium dichromate, all fish should survive (that is $T_F = 1$).

9.1.2 All the conditions defined in the test method are fulfilled.

10 REPORT

10.1 The test report shall include the following information.

10.1.1 The specification of the test effluent and full information for identification of test samples.

10.1.2 Any deviation from the procedure specified in this standard and the reason for this, including a description of the circumstances which could have influenced the results.

ANNEX A

(Foreword)

COMMITTEE COMPOSITION

Water Environment Protection Sectional Committee, CHD 12

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DR V. K. VERMA	Punjab Pollution Control Board, Punjab
SHRI M. S. DHINGRA (<i>Alternate</i>)	The Dharamsi Morarji Chemical Co Ltd, Mumbai
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REPRESENTATIVE	
DR DIPAK CHAKRABORTY	
DR UJJAL MUKHERJEE (<i>Alternate</i>)	
REPRESENTATIVE	

(Continued on page 5)

(Continued from page 4)

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Director (Chem)

Representing

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National Productivity Council, New Delhi
Department of Rural Development, New Delhi
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Directorate General of Health Services, New Delhi
Ministry of Environment & Forests, New Delhi
Water Technology Engineering Division, Nagpur
RDSO, Ministry of Railways, Lucknow
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DR R. K. SINGH
Additional Director (Chem), BIS

